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(54) Title: CONTROL OF GENE TRANSCRIPTION

(57) Abstract

The plant gene TFIID encodes DNA-binding protein necessary for the transcription of all structural genes. Down-regulation of the expression of TFIID, by transformation with a sense or antisense copy of the TFIID gene, prevents expression of the gene, leads to a failure of transcription and, consequently, cell death. Such down-regulation may be targeted to particular cell types or to particular development stages by the selection of suitable promoters. Since TFIID is expressed in all celis at all times, its promoter is truly constitutive in effect and may be used to drive constitutive expression of foreign genes placed downstream thereof.

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CONTROL OF GENE TRANSCRIPTION

The present invention relates to a gene which encodes a factor required for transcription in plant cells and a method for the control of transcription using that gene.

Development, form and function of plants are brought about by the timed and co-ordinated expression of many thousands of genes. These genes are expressed in specific tissues, at different times, for different periods of time, in response to different end signals. The activation and repression of gene expression is controlled through the interaction of protein factors with specific DNA sequences associated with genes. Factors controlling the initiation of transcription of protein coding genes by RNA polymerase II (B) can be divided into two groups, general factors and activators. General transcription factors are responsible for assembly of the pre-initiation complex at the 'TATA' box, and accurate basal level transcription initiation. Activators are an heterogeneous class of sequence-specific DNA binding proteins that interact with the pre-initiation complex to bring about regulated high level transcription of the associated gene. Interaction of activators with the pre-initiation complex is presumed to be an important level of control for gene expression.

Assay systems for the analysis of transcription initiation in-vitro have been described for animals and plants. These contain general transcription factors and RNA polymerase II derived from specific organs and cell types, and have been used in the analysis of both cis-sequences and activators. An important feature of such systems is the higher level regulated transcription which can be obtained

in the presence of added activator protein , indicating that the activator interacts with one or several components of the general transcription machinery. In animal and yeast systems many of these general factors have been identified and purified. From such studies several consistent features of the number and type of factors have emerged, as has their order of assembly on the DNA. The most important of the general factors is Transcription Factor IID (TFIID), necessary for template commitment (as it binds to the TATA box) which may interact with at least one activator protein via a 'coactivator'. Following the binding of TFIID, other factors (TFIIA/G, TFIIB, RNA polymerase 2 and TFIIE/F) are recruited to form the fully assembled complex. In addition to TFIID, TFIIB has recently been shown to directly interact with activator proteins in animal systems. Recent analysis of TFIID from Hela cells has revealed that the endogenous factor is composed of a single DNA-binding protein (the TATA-binding protein,TBP) and several other unrelated components. TFIID TBP-component is composed of both conversed and species-specific elements. Homology between TBPs is at least 80% or greater in the carboxy-terminal 200 amino acids. The amino terminal ends of TBPs are highly disparate, and were initially presumed to play some role in species-specific interactions that regulate transcription initiation. Although it is possible that this does occur other evidence has pointed to functional differences being localised to the highly conserved carboxy-terminal region. Reports to date have focused on analysis of the physical requirements of protein structure for TBP function.

An object of the present invention is to provide means for controlling gene transcription in plants.

According to the present invention there is provided a cDNA having the sequence given in Figure 1 herewith and variations therein permitted by the degeneracy of the

genetic code, and any equivalent genomic sequence encoding TFIID TBP-component to which said cDNA hybridises.

The invention further provides the cDNA located on plasmid pTFIID which has been deposited, in an Escherichia coli, strain DH5α, host, under the terms of the Budapest Treaty on the Deposit of Microorganisms for Patent Purposes, with the National Collection of Industrial and Marine Bacteria, Aberdeen, United Kingdom, on 19th December 1991 under the Accession Number NCIB 40467.

Also, the invention provides a method of inhibiting the growth of a plant cell comprising stably incorporating into the genome of the said plant by transformation a full or partial length copy of a nucleotide which is antisense to the cDNA defined hereinabove or its genomic equivalent.

Such inhibition may also be obtained by stably incorporating into the genome of the said cell by transformation a full or partial length copy of the cDNA defined hereinabove or its genomic equivalent.

Further according to the invention there is provided a method for the isolation of the TFIID promoter, comprising probing a genomic library with the cDNA of the invention, isolating a genomic sequence which hybridises to the said cDNA and recovering from the isolated genomic sequence th promoter region lying upstream of the TFIID gene.

The invention thus also provides the promoter sequence of the TFIID gene.

Because the TFIID is constitutively expressed, the invention also provides a method for the induction of constitutive gene expression, comprising placing a selected gene of interest under control of the promoter of the TFIID gene.

Therefore, the invention further provides a recombinant gene construct comprising in sequence a TFIID promoter, a selected gene for expression and a transcription terminator

region.

The TFIID gene can be used as a target to inhibit growth of a plant cell by several methods. In addition the gene sequences can be used to isolate the gene in question which is a truly constitutive gene. TFIID is a DNA-binding protein or factor which binds to DNA sequences found on plant promoters such as the "TATA box". Interaction of other factors involved with the initiation of transcription or the enhancement of transcription with the factor is specifically required for gene expression in plants.

The cDNA can be used to isolate the gene encoding it. This is described in detail below. This gene is special in that due to the requirement that it be expressed in all tissues of a plant at all times, it is expressed constitutively. Therefore the promoter controlling it is must be a truly constitutive promoter.

In addition, inhibition of the expression of this gene in specific tissues or at a specific stage during plant development is expected to lead to the inhibition of plant development at that given time. This can be used to inhibit flowering, pollen formation, embryo development and seed formation among others.

The present invention also provides a plant having stably incorporated in its genome by transformation a gene construct carrying a tissue-specific or a development-specific promoter which operates in the cells of the target plant tissue and a DNA binding protein gene construct which is capable, when expressed, of inhibiting transcription in the cells of the said target tissue resulting in death of the cells.

One preferred application of this invention is in the creation of male sterile plants for use in the production of hybrids. Our International Patent application WO 90/00110, incorporated herein by reference, relates to the production

of such male sterile plant lines. Essentially our invention, the subject of that International Application, is a plant gene construct comprising a disrupter gene encoding a protein which is capable of disrupting the biogenesis of viable pollen, and a gene regulatory sequence and which includes a promoter sequence inducible by external application of an exogenous chemical inducer to a plant containing the construct. In a specific embodiment, the construct comprises:

(a) a first gene promoter sequence responsive to the presence or absence of an exogenous chemical inducer,

(b) a gene encoding a repressor protein under control of the said first promoter sequence;

(c) an operator sequence responsive to the said repressor protein expressed by the repressor protein gene;

(d) a male flower specific gene promoter sequence expressible only in male parts of a plant; and,

(e) a gene encoding a disrupter protein capable of disrupting biogenesis of viable pollen;

whereby the presence or absence of the exogenous chemical inducer in the plant enables selection of male fertility or sterility.

Examples of the disrupter protein are; the mammalian uncoupling protein (UCP) gene, a mutated form of the gene for the β-subunit of F_1-ATPase which has sequences added or deleted such that these changes result in the retention of the ability to assemble with other subunits but interf re with function as an ATP synthase, a mutated, synthetic form of the oli 1 gene encoding subunit 9 of the F_o-ATPase, a mutated form of a mitochondrial transit pre-sequence which malfunctions during transfer resulting in the disruption of protein transport to mitochondria, and, a gene construct carrying a fusion between the β-subunit gene from yeast and the β-glactosidase gene from E. coli, resulting in

expression of a disrupting fusion protein.

The present invention provides an alternative means of disrupting pollen formation comprising inhibition of the TFIID gene.

The invention also provides a plant, particularly a monocotyledonous plant, and more particularly a corn plant, having stably incorporated within its genome a gene construct carrying a tissue-specific promoter which operates in the cells of the said target gene constructs may be used as a means of inhibiting cell growth in a range of organisms from simple unicells to complex multicellular organisms such as plants and animals. By the use of tissue- or cell-specific promoters, particular cells or tissue may be targeted and destroyed within complex organisms. One particular application could be the destruction of cells essential for male flower development, leading to male sterility or the inhibition of seed development leading to plants which do not carry seeds.

The invention therefore provides a method of preventing or inhibiting growth and development of plant cells based on gene constructs which inhibit transcription and gene expression. The technique has wide application in a number of crops where inhibition of particular cells or tissue is required.

Of particular interest is the inhibition of male fertility in maize for the production of F1 hybrids in situ.

The method of transformation is not particularly germane to this invention and any method suitable for the target plant may be employed. Transgenic plants are obtained by regeneration from the transformed cells. Numerous transformation procedures are known from the literature such as agroinfection using Agrobacterium tumefaciens or its Ti plasmid, electroporation, microinjection of plant cells and protoplasts,

microprojectile transformation and pollen tube transformation, to mention but a few. Reference may be made to the literature for full details of the known methods.

The invention will now be described in following Examples.

EXAMPLE 1

cDNA library synthesis and screening

A cDNA library was constructed in Lambda gt-11 using polyadenylated RNA obtained from young potato (Solanum tuberosum L.cv. Desiree) tubers according to the manufacturers recommendations (Amersham). The cDNA library (10^6 recombinant phage) was screened with a TBP cDNA clone from Arabidopsis thaliana L, and the inserts from hybridising clones were sub-cloned into pUBS-3. The complete DNA sequence of cDNA inserts was determined on both strands by sequencing of plasmid DNA, and DNA and derived protein sequence was analysed using Mac Vector DNA sequence analysis software.

A TBP cDNA clone from Arabidopsis thaliana L was used to screen a cDNA library from potato tubers. From an initial screen of 10^6 clones from an amplified portion of this library one clone was isolated (ST-1), and from a subsequent screen of 10^6 clones from the primary library a further three clones were isolated (ST-2, ST-3, ST-4). The complete nucleotide sequence of ST-2 (representing the largest spliced cDNA insert) was determined (Figure 1). This DNA sequence was compared to those available in the EMBL-database and indicated a high level of homology between ST-2 and TBPs from both human and yeast sources.

The cDNA ST-2 contains an open reading from corresponding to a protein of molecular weight 22 kDa, similar to the molecular weights reported for TBP from Arabidopsis thaliana L. Comparison of this protein sequence with predicted TBP from other species shows that the protein

encoded by ST-2 has a high degree of similarity to the putative TBPs 1 and 2 from Arabidopsis thaliana L (Figure 2). These proteins are of identical length (200 amino-acids), and are 94.5% and 94% homologous respectively to the potato protein. Potato TBP also shares over 80% homology with the carboxy-terminal region of TBP from humans, yeast and Drosophila melanogaster.

EXAMPLE 2

Production and analysis of in-vitro synthesised TBP:

A nested set of 3' truncations of the TBP cDNA were constructed by digestion of plasmids with suitable restriction enzymes. Plasmid DNAs containing the full-length TBP cDNAs were linearised at the 3' end of the cDNA by restriction digestion. RNA was synthesised in vitro from T7 RNA polymerase promoter located 5' to the cDNA, purified, and used to program a rabbit reticulocyte in-vitro translation reaction. In-vitro synthesised protein was used in electrophoretic mobility shift assays (EMSAs), using an end-labelled 360 base-pair DNA fragment (position -340 to +20) from the potato patatin class 1 promoter (21) as a specific probe. Reactions were carried out in 10μl volumes containing 0.1μl reticulocyte-lysate translation product as previously described (20) using radiolabelled probe DNA (290pM, 4000cpm) and 333ng (63.4nM) polydGdC.dGdC (Pharmacia) at 25°C for 30 minutes unless otherwise stated. Protein/DNA complexes were resolved on native 4% polyacrylamide gels.

The functional requirements of cloned potato TBP were determined using in-vitro synthesised protein. pST-2 was used to produce potato TBP protein in-vitro by transcription of the linearised plasmid and subsequent translation of RNA (Figure 3). Carboxy-terminal truncated versions of ST-2 protein were produced following linearisation of plasmid DNA with restriction enzymes cutting within the predicted open

reading frame. ST-2 protein and truncated derivatives synthesised in this way were tested for DNA-binding specificity using the electrophoretic mobility shift assay (EMSAs 22,26). In-vitro translation of RNA from linearised plasmids gave proteins of the correct predicted molecular weights. The full length protein was capable of DNA binding to the potato patatin class-1 gene promoter. All truncation derivatives of ST-2 TBP were however not able to support DNA binding or transcription enhancement, within a 10-fold range of protein concentration of that shown (data not shown).

EXAMPLE 3

Biochemical analysis of TBP DNA binding

Double-stranded oligonucleotides used in competition experiments were of the following structure (top-strand shown):

1. Wild type 5'tcgacTTGTTTACGTGCCTATATATACCATGCTTG TTATATGCTCg-3'

2. mutant 5'-tcgacTTGTTTACGTGCCTAGGGGTACCATGCTTG TTATATGCTCg-3'.

Nucleotides which are shown in lower case letters indicate changes from the wild type patatin TATA sequence motif (underlined), and lower case nucleotides indicate and added restriction enzyme site. For heat stability experiments protein preparations were heat treated for 10 minutes, and bound DNA was separated from free DNA at increasing times thereafter. The relative affinity ration of potato TBP for polydGdC.GdC and the patatin promoter was calculated as previously described . The Apparent Equilibrium constant (K_{app}), C_O (the number of binding sites in the protein preparation(K_s and K_n (the specific and non-specific equilibrium constants) were calculated as previously described. [$D_n°$] values (expressed as moles polydGdC.dGdC base-pairs per litre) were obtained from experiments in which the amount of polydGdC.dGdC was varied

and patatin promoter fragment kept constant. EMSAs were quantified by cutting out from the dried gel and Cherenkov counting. A standard calibration curve indicated that the relationship between input DNA and counted fractions was linear. All experiments were conducted at least three times, and at least two separate protein/RNA preparations were used. Results shown are the average of three separate experiments.

Biochemical characteristics of plant TBP activity were analysed using the full-length ST-2 protein (Figure 4). Analysis of the physical properties of the ST-2 protein-DNA interaction indicated that maximum DNA binding occurred at a KCl concentration of 200mM, and $MgCl_2$ of 5mM. DTT did not influence DNA binding capacity under optimised conditions (data not shown). TBP was shown to be heat-labile, more than 80% of the available activity being destroyed by a 10 -minute incubation at 45°C. Maximum DNA binding occurred between 25-30°C, the apparent equilibrium constant after 30 minutes incubation being reduced substantially at lower and higher temperatures. On and off-rate measurements for the protein-DNA complex indicated that both association and dissociation of TBP with DNA is slow (saturation for association occurring after 30-40 minutes, and only 20% TBP being dissociated after an additional 20 minutes in the presence of excess specific competitor DNA).

The copolymer polyGdC.dGdC (used as non-specific DNA in EMSA experiments) was a very inefficient competitor for TBP binding to the patatin promoter (Figure 4). Specificity of DNA binding for the TATA box was demonstrated in competition experiments in which an oligonucleotide containing the wild type TATA motif from the patatin gene (see Figure 3) was shown to compete effectively with the patatin gene promoter for ST-2 binding, whereas an oligonucleotide containing a mutated TATATATA box (replaced with TAGGGGTA) was competed

at a very much lower level. The relative affinity of the wild type TATA-box DNA sequence was measured with respect to the mutated sequence and polydGdC.dGd.C by analysis of the levels of competitor DNA that reduce TBP DNA binding 50% (relative affinity ratio; 22,26). Results indicate that potato TBP binds the copolymer polydGdC.dGdC with 1.77×10^6-times lower affinity.

The apparent equilibrium constant (K_{app}) under optimised reaction conditions for potato TBP binding to the patatin promoter TATA-box was calculated from the binding data (Figure 5d), and in four independent experiments gave a value 2.4×10^9 $M^{-1} \pm 1.1 \times 10^{11}$. The values of K_s (the specific equilibrium constant), and the K_n (the non-specific equilibrium constant) were calculated from Figure 5e and f, and were 5×10^9 M^{-1} and 3.65×10^4 M^{-1} respectively.

EXAMPLE 4

Isolation of the gene encoding TFIID

In all non-plant organisms investigated so far TBP is encoded as a single gene. In contrast, TBP from Arabidopsis is present in at least two copies. To determine the representation of ST-2 TBP sequences in the potato genome, genomic southern experiment were performed with potato DMS. The probe (pst-2) hybridised strongly to a single fragment of potato DNA when using restriction enzyme BglI and to two DNA fragments when using EcoRI. Very much weaker hybridisation is also visible to one additional DNA fragment in the BglI digested DNA upon longer exposure of the filter to film. The clone ST-2 does not contain any EcoRI sites, and only a single BglII site at position 35. Analysis of the sequence of an incomplete partially spliced potato TBP cDNA clone ST-1 did not reveal any additional EcoRI or BglII restriction sites. These data suggest that in potato TBP is encoded in the genome as a low copy number sequence, possibly as a single gene per haploid genome.

A genomic library of potato was screened using the cDNA clone. A genomic clone can be isolated using this approach.

International Applicau n N : PCT/

MICROORGANISMS

Optional Sheet in connection with the microorganism referred to on page 3, line 3-9 of the description :

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Further deposits are identified on an additional sheet ☐ :

Name of depositary institution :

NATIONAL COLLECTION OF INDUSTRIAL & MARINE BACTERIA

Address of depositary institution (including postal code and country) :

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Aberdeen AB2 1RY
United Kingdom

Date of deposit :	Accession Number :
19th December 1991	NCIMB 40467

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(Authorized Officer)

☐ The date of receipt (from the applicant) by the International Bureau :

was

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CLAIMS

1. A cDNA having the sequence given in Figure 1 herewith and variations therein permitted by the degeneracy of the genetic code, and any equivalent genomic sequence encoding TFIID TBP-component to which said cDNA hybridises.

2. The cDNA located on plasmid pTFIID which has been deposited, in an Escherichia coli, strain DH5α, host, under the terms of the Budapest Treaty on the Deposit of Microorganisms for Patent Purposes, with the National Collection of Industrial and Marine Bacteria, Aberdeen, United Kingdom, on 19th December 1991 under the Accession Number NCIB 40467.

3. A method of inhibiting the growth of a plant cell comprising stably incorporating into the genome of the said plant by transformation a full or partial length copy of a nucleotide which is antisense to the cDNA claimed in claim 1 or its genomic equivalent.

4. A method of inhibiting the growth of a plant cell comprising stably incorporating into the genome of the said cell by transformation a full or partial length copy of the cDNA claimed in claim 1 or its genomic equivalent.

5. A method for the isolation of the TFIID promoter, comprising probing a genomic library with th cDNA claimed in claim 1, isolating a genomic sequence which hybridises to the said cDNA and recovering from the isolated genomic sequence th promoter region lying upstream of the TFIID gene.

6. The promoter sequence of the TFIID gene.

7. A method for the induction of constitutive gene expression, comprising placing a selected gene of interest under control of the promoter claimed in claim 6.

8. A recombinant gene construct comprising in sequence a TFIID promoter, a selected gene for expression and a transcription terminator region.

9. A plant gene construct comprising a disrupter gene encoding a protein which is capable of disrupting the biogenesis of viable pollen, and a gene regulatory sequence and which includes a promoter sequence inducible by external application of an exogenous chemical inducer to a plant containing the construct in which the said disruption of pollen biogenesis is achieved by the method claimed in claim 3 or claim 4.

FIG. 1 (1/2)

```
ATATATATATTCATCCTTCCTTGTCGCTCTACAAAGATCTTCTTCAT
ATTATTTCTTTACAACAAGCAAAATCCTCCAATTTCAGCAAACCCTA
TAGGTCAAAAGAGGAATGGCAGATCAGGGATTAGAGGGGAGCCAGCC
                  M  A  D  Q  G  L  E  G  S  Q  P

CAGAATATTGTCTCAACGGTTAATTTGGACTGCAAGTTGGACCTGAA
 Q  N  I  V  S  T  V  N  L  D  C  K  L  D  L  K

CGTTTTGCTGCAGTGATCATGAGAATTAGAGAACCAAAAACTACAGC
 R  F  A  A  V  I  M  R  I  R  E  P  K  T  T  A
    F           I     R           P  K
AGTGAACAACAGTCAAAGTTGGCAGCCCCGAAATATGCTAGAATCAT
 S  E  Q  Q  S  K  L  A  A  P  K  Y  A  R  I  I

CAGAATATAGTTGGTTCTTGTGATGTTAAATTTCCTATTCGACTTGA
 Q  N  I  V  G  S  C  D  V  K  F  P  I  R  L  E

GAACTATTTCCTGGATTAATATATCGCATGAAACAACCAAAAATAGT
 E  L  F  P  G  L  I  Y  R  M  K  Q  P  K  I  V

AAGGTTAGAGATGAGACATATACTGCCTTTGAGAACATATACCCAGT
 K  V  R  D  E  T  Y  T  A  F  E  N  I  Y  P  V

GACTGTGGAGCATCTGCTATGACTAACTGCAAGGGGTGGTTCAAAAT
ATCAGGCATCGGCTTTTGTGGAAGGTTTGTTTAAGTTACAATTGATA
TGTAGCATATCATAAAATTTCAATTTAGGACCAAAAAAAGCCAAACT
CTTGATAGCGGCCTTTTGGTTTACTTTTCCTCAAAAAAAA
```

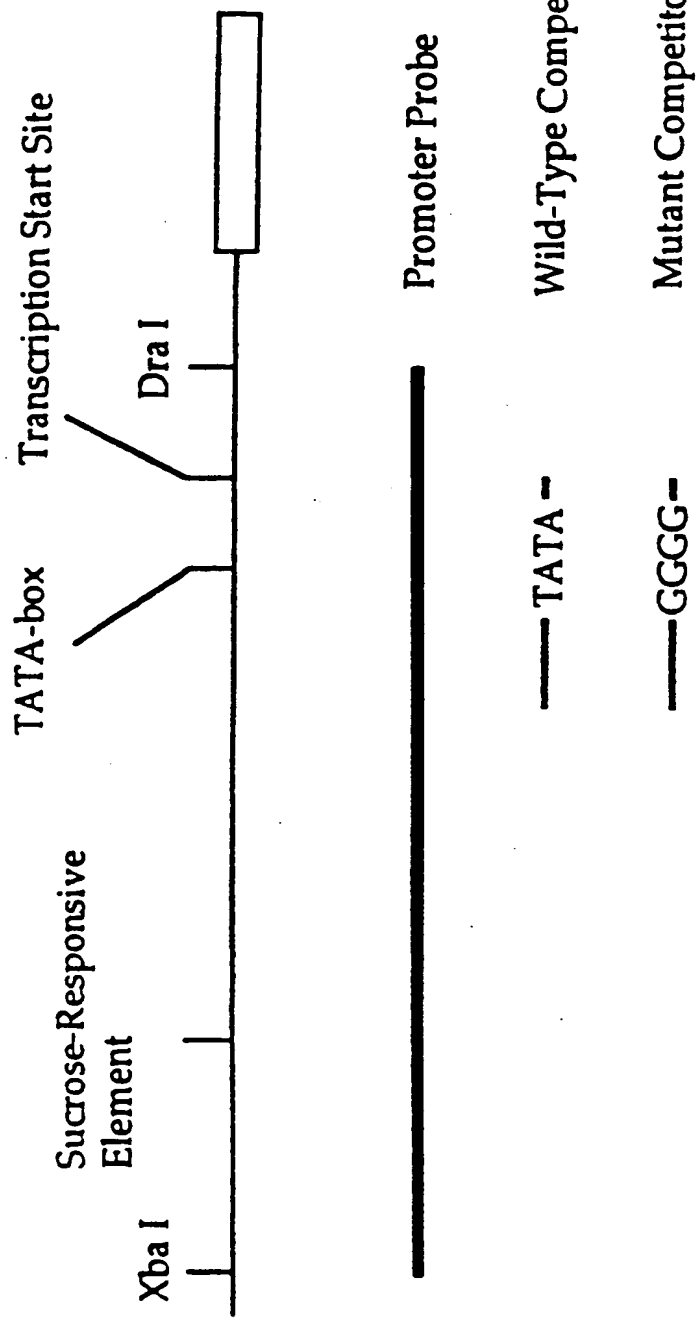
FIG. 1 (2/2)

```
ATAAAAGAATCCTTCTTTCAAATTCTTTACCCGCTACAAATTC 90
GTTTTAGATTTTCAGTTTAAAGAGTTTTGTGTACGGAAGGGAC 180
AGTGGATCTTACCAAGCACCCTTCTGGAATAGTCCCTACTCTG 270
  V  D  L  T  K  H  P  S  G  I  V  P  T  L  25

AGCTATTGCACTTCAAGCTCGAAATGCAGAGTACAATCCGAAG 360
  A  I  A  L  Q  A  R  N  A  E  Y  N  P  K  55
                        (143) Y     P
GTTGATTTTCGCTTCTGGGAAAATGGTTTGTACTGGAGCCAAA 450
  L  I  F  A  S  G  K  M  V  C  T  G  A  K  85
  L  I  F     S  G  K     V     T  G  A  K
TCAAAAGCTTGGTTTTCCAGCCAAGTTTAAGGATTTTAAAATC 540
  Q  K  L  G  F  P  A  K  F  K  D  F  K  I  115

AGGCCTTGCATATGCCCACGGTGCTTTCTCAAGTTACGAGCCA 630
  G  L  A  Y  A  H  G  A  F  S  S  Y  E  P  145

GCTGCTTATTTTTGTTTCTGGGAAAATTGTCATCACAGGAGCC 720
  L  L  I  F  V  S  G  K  I  V  I  T  G  A  175

TCTTACCGAGTTCAGGAAGAATCAGCAATGAATTGATTTGATG 810
  L  T  E  F  R  K  N  Q  Q                   200

TTGGAGACTTGTTTGTTTTTTCTTAATTGTAGATATTCTATAA 900
GGTTGGAAGATTAGCAAGATGGGGATAACTGTGTTACTTCACC 990
GGTCATGGGTTCCAAATTTATTGTACCTGTTGAGTCTATAATG 1080
                                             1120
```

FIG.2

```
1
MADQGLEGSQPVDLTKHPSGIVPTLQNIVSTVNLDCKLDLKAIALQARNAEYNPKRFAAVIMRIREPKTT  Potato
     T                                                                    Arabidopsis 1
 T       N     S                                                          Arabidopsis 2
EE IKRATPESEKD SAT           A  T G R    TV  H                           Yeast
QTMGPSTPMT ATPGSADP    Q          C      K   H                      R     Drosophila
SPMTPMTPIT ATPASES     Q          G      T   R                      R     Human
71
ALIFASGKMVCTGAKSEQQSKLAARKYARIIQKLGFPAKFKDFKIQNIVGSCDVKFPIRLEGLAYAHGAF  Potato
                  HL           V                                  S S     Arabidopsis 1
                  DF  M        V                                  S A     Arabidopsis 2
         V        DD    S         I  A   T                       FS  T   Yeast
    S             DD R                    L       M               VLT CN  Drosophila
    S             E  R         VV         L       M               VLT QQ  Human
141
SSYEPELFPGLIYRMKQPKIVLLIFVSGKIVITGAKVRDETYTAFENIYPVLTEFRKNQQ  Potato
                 L                      M E              R    V   Arabidopsis 1
                 V                      M     K          S    I   Arabidopsis 2
                VK                        L    Q E I Q   A    S    M..  Yeast
                VR R                   V  L      Q I D  DK F I KK K QS.  Drosophila
                IK R                   V  L      A I E       I KG   TT.  Human
```

FIG. 3



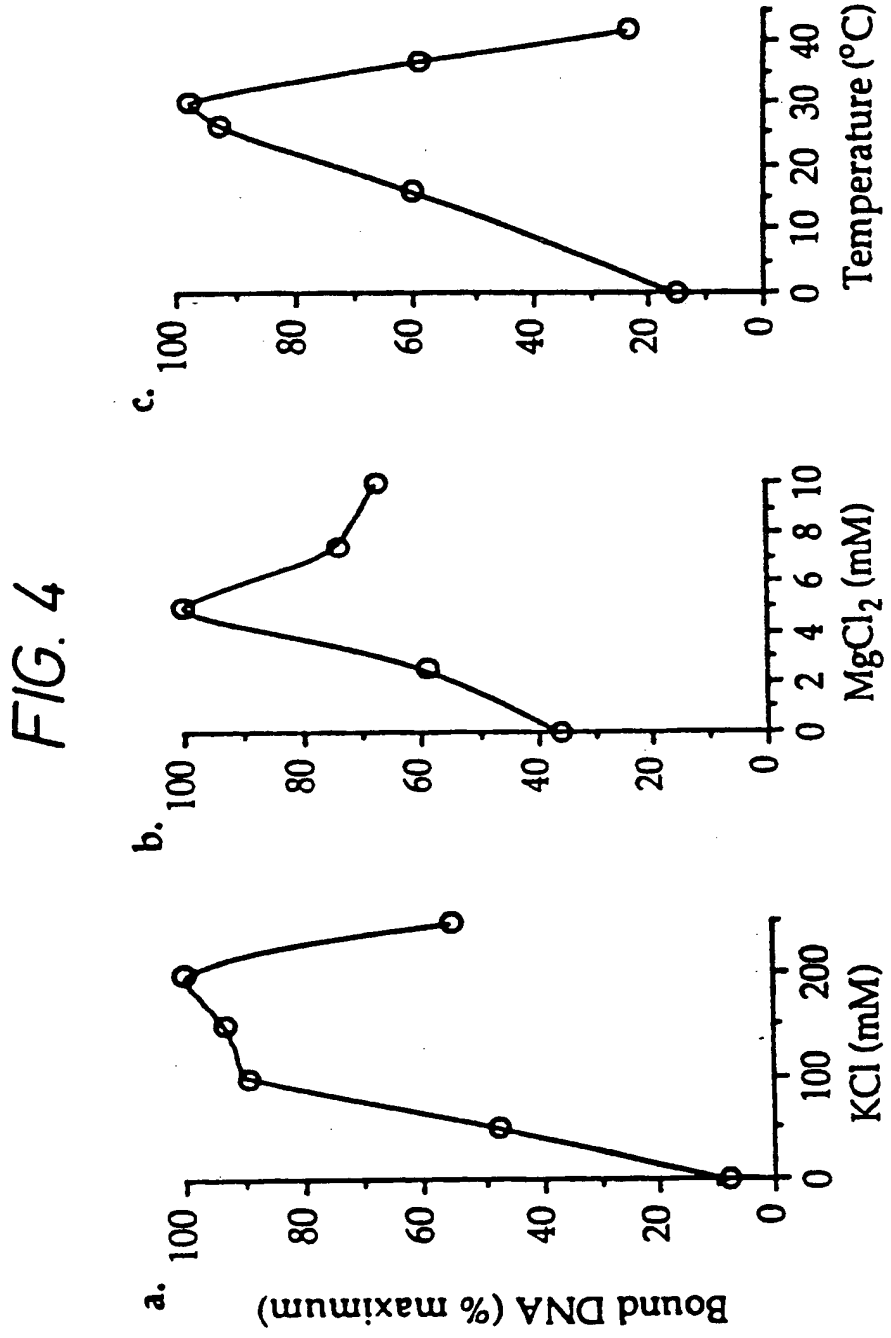

Bound DNA (% maximum)
a.
b.
c.
0
20
40
60
80
100
0 100 200
KCl (mM)
0 2 4 6 8 10
$MgCl_2$ (mM)
0 10 20 30 40
Temperature (°C)

FIG. 4

FIG. 4

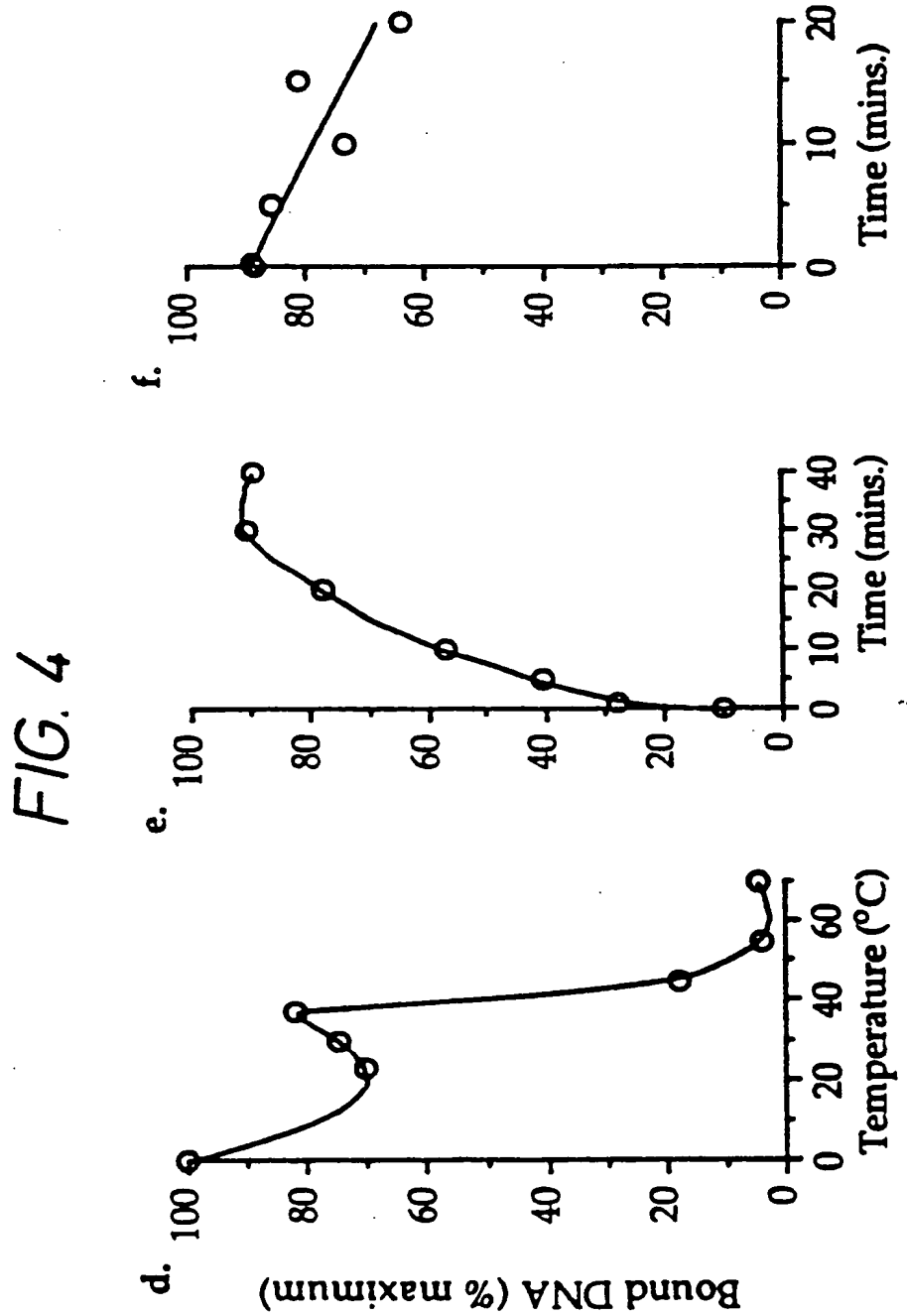

Bound DNA (% maximum)
d.
e.
f.
100
80
60
40
20
0
0 20 40 60
Temperature (°C)
0 10 20 30 40
Time (mins.)
0 10 20
Time (mins.)

FIG. 5

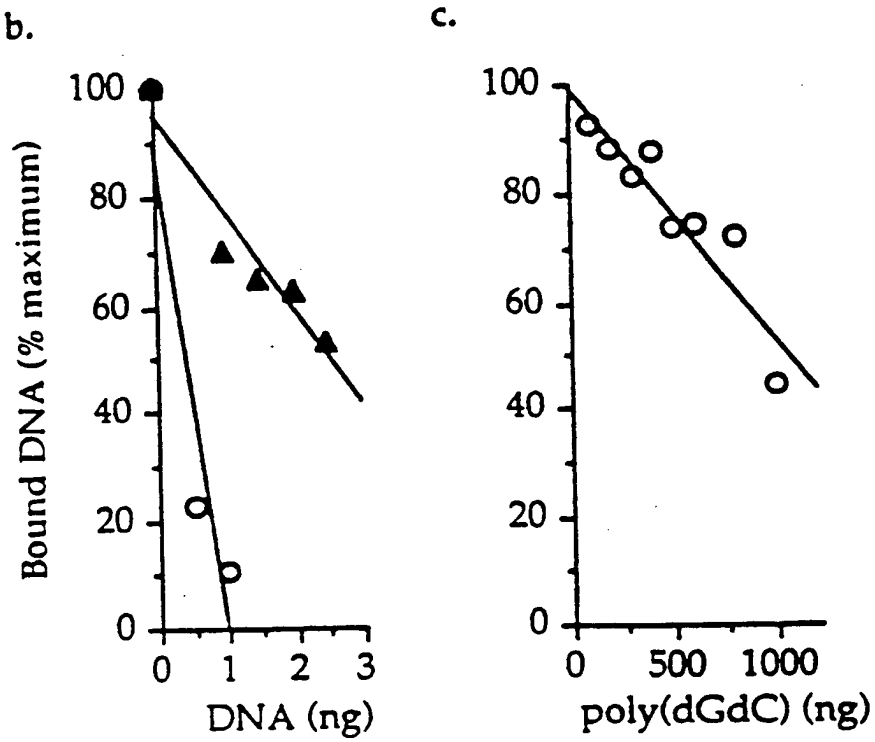
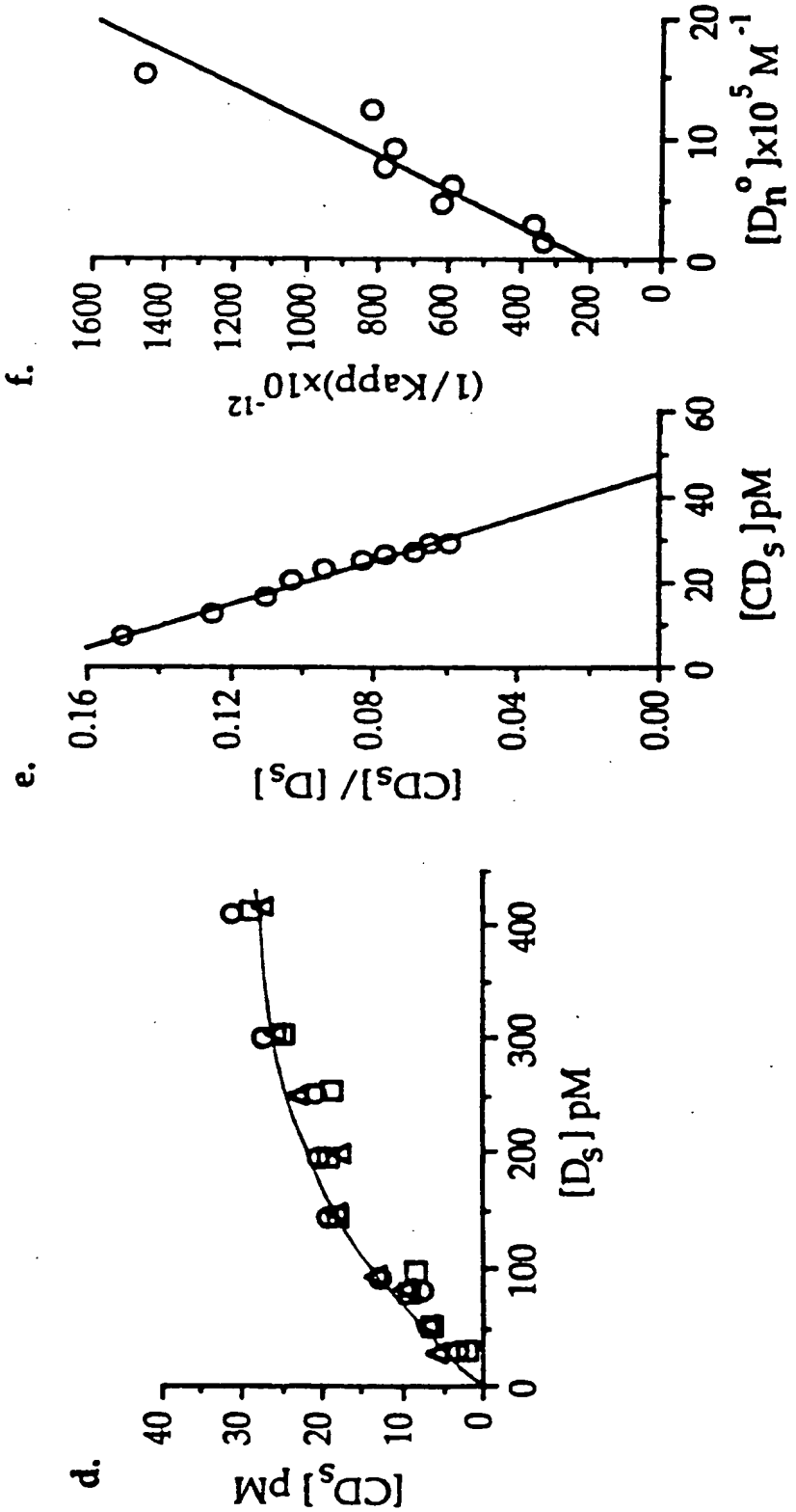

b.
c.
Bound DNA (% maximum)
100
80
60
40
20
0
0 1 2 3
DNA (ng)
0 500 1000
poly(dGdC) (ng)

FIG. 5



INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 93/00137

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C12N15/82; C12N15/29; C12N15/11

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C12N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	EMBL SEQUENCE DATABASE, HEIDELBERG, FRG. RELEASE 30, 31 OCT. 1991. ACCESSION NO. X62494 TATA-BINDING PROTEIN.	1,2

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
24 MAY 1993	21-06-1993
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	MADDOX A.D.

Form PCT/ISA/210 (second sheet) (January 1985)